

Small 6q16.1 Deletions Encompassing *POU3F2* Cause Susceptibility to Obesity and Variable Developmental Delay with Intellectual Disability

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Genetic studies of intellectual disability and identification of monogenic causes of obesity in humans have made immense contribution toward the understanding of the brain and control of body mass. The leptin > melanocortin > SIM1 pathway is dysregulated in multiple monogenic human obesity syndromes but its downstream targets are still unknown. In ten individuals from six families, with overlapping 6q16.1 deletions, we describe a disorder of variable developmental delay, intellectual disability, and susceptibility to obesity and hyperphagia. The 6q16.1 deletions segregated with the phenotype in multiplex families and were shown to be de novo in four families, and there was dramatic phenotypic overlap among affected individuals who were independently ascertained without bias from clinical features. Analysis of the deletions revealed a ~350 kb critical region on chromosome 6q16.1 that encompasses a gene for proneuronal transcription factor *POU3F2*, which is important for hypothalamic development and function. Using morpholino and mutant zebrafish models, we show that *POU3F2* lies downstream of *SIM1* and controls oxytocin expression in the hypothalamic neuroendocrine preoptic area. We show that this finding is consistent with the expression patterns of *POU3F2* and related genes in the human brain. Our work helps to further delineate the neuro-endocrine control of energy balance/body mass and demonstrates that this molecular pathway is conserved across multiple species.

Intellectual disability has an estimated prevalence of 1.5%–2.0%¹ and is a genetically and phenotypically heterogeneous group of disorders. Studies of genetic causes of intellectual disability have made immense contributions toward our understanding of the human brain. Obesity and related co-morbidities are a major public health concern across the world.² Understanding the control mechanisms of body mass is a fundamental question for biology and an important area for research.

Rare copy-number variations (CNVs) are linked with a range of phenotypes and are a particularly well-recognized cause of developmental disorders and intellectual disability.^{3,4} Additionally, rare CNVs can provide insights into the single-gene causes of human disorders^{5,6} and can provide clues toward the genetic basis and molecular mechanisms of commoner complex conditions and traits^{7,8} including obesity.^{9–14} Here, we describe a study of small overlapping 6q16.1 deletions in individuals with

variable developmental delay, intellectual disability, and susceptibility to obesity and hyperphagia along with extended analyses that define the likely critical gene for the phenotype and its role in neuro-endocrine control of energy balance and body mass.

Ethics approval for the study was obtained from the NHS ethics committee (11/H1003/3) and the University of Manchester. Informed consent was taken from all participants recruited into the study from the Manchester Centre for Genomic Medicine. Other participants provided consent to publish their data to the recruiting clinician.

We identified a family (referred to as family 1) with four members, most of whom were affected with neonatal hypotonia, gross motor delay, speech delay, intellectual disability, behavioral problems, obesity, and hyperphagia with onset from mid-childhood (Figure 1A). Their clinical features are summarized in Table 1. Family history suggested an autosomal-dominant inheritance pattern, and

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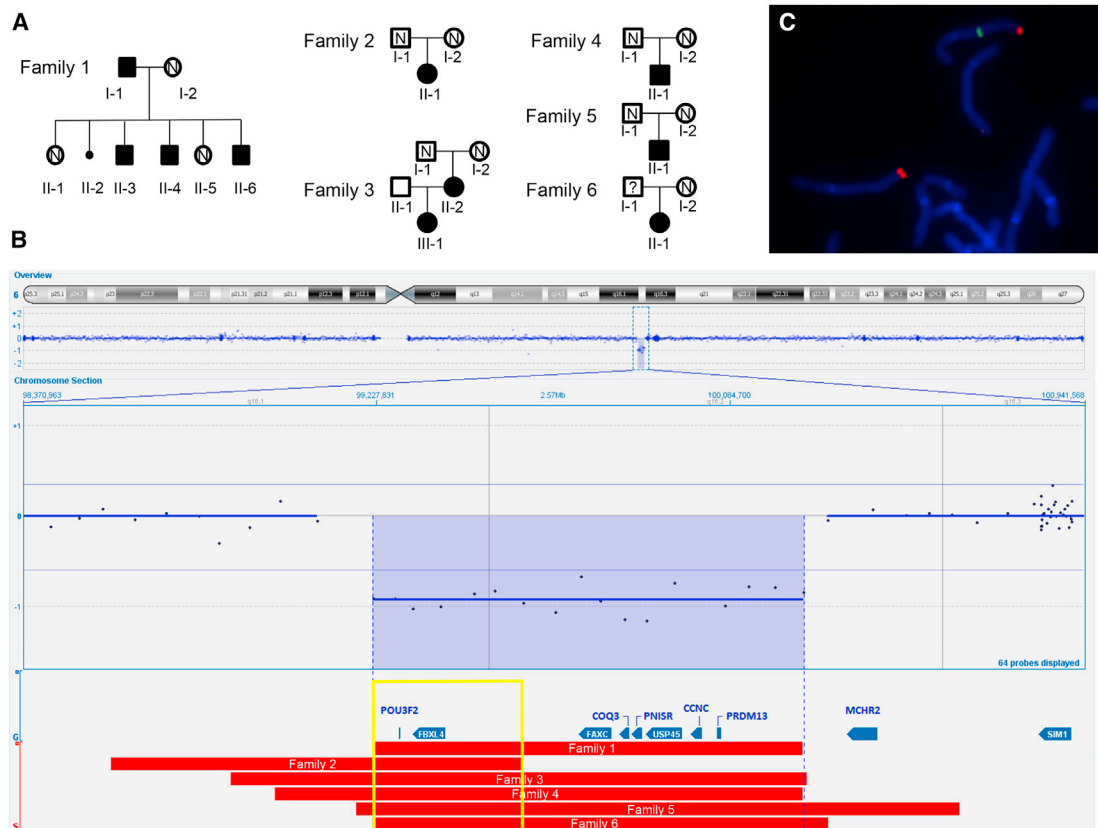


Figure 1. Results of Clinical Genetic Studies

(A) Pedigrees of families. Array comparative genomic hybridization (aCGH) on a DNA sample from individual II-4 of family 1 revealed a 1–1.2 Mb heterozygous deletion on chromosome 6q16.1q16.2 that segregated with the phenotype in the family. We interrogated the local clinical cytogenetics databases of our collaborators for <2 Mb 6q16 deletions that do not include *SIM1* and identified six additional individuals from five families (family 2–6). In four individuals, deletions were proven to have arisen de novo. One individual in family 3 had inherited the deletion from her affected mother. Standard symbols have been used to draw the pedigrees. Dark squares represent affected individuals who were found to have 6q16 deletion. Squares or circles with “N” denote individuals who were tested and found not to have the familial 6q16 deletion. “?” denote individuals whose genotype information is not available.

(B) Results of copy-number analysis. The top panel represents the chromosome bands with the copy-number state of the corresponding hybridized probes from the aCGH results of individual II-4 of family 1. The middle panel focuses on the 6q16 region. The horizontal red bars in the bottom panel show the minimum extent of the microdeletions (in hg19 build) in all five families. The bottom panel is annotated with respective gene loci. The yellow box circumscribes the maximum common overlapping region of the deletion in the five families.

(C) Metaphase fluorescent in situ hybridization (FISH) from individual II-4 from family 1. FISH was undertaken with spectrum green fluorophore-prelabeled RP11-290C18 BAC probe (The Centre for Applied Genomics, Toronto, Canada) which maps to the 6q16.2 region (chr6: 99,813,064–99,990,209). A spectrum orange fluorophore-prelabeled 6q subtelomeric probe (Abbott Molecular) was used as a control. The FISH independently confirmed the heterozygous 6q16.2 deletion in this individual.

Prader Willi syndrome (PW syndrome or PWS) (MIM: 176270) was ruled out via methylation-specific multiplex ligand probe amplification. An array comparative genomic hybridization (aCGH) was performed on a DNA sample from individual II-4 via CytoSure ISCAv2 (8x60k) microarray (Oxford Gene Technology) according to the manufacturer’s protocol. CytoSure Interpret v3.4.3 software was used for data analysis, and copy-number aberrations were detected using a minimum of four markers per segment with abnormal log2 ratios (~180 kb backbone and ~15 kb targeted resolution). This revealed a 1–1.2 Mb deletion on chromosome 6q16.1q16.2 (chr6: 99,218,535–100,260,996 in hg19) (DECIPHER: 265018) (Figure 1B). The deletion was absent in the Database of Genomic Variants (DGV) and in more than 6,000 local controls. Metaphase fluores-

cent in situ hybridization (FISH) analysis performed on lymphocyte cell suspensions using standard protocols independently confirmed the 6q16 deletion in II-4 (Figure 1C) and in all affected family members (II-3, II-6, and I-1). The deletion was absent in all the unaffected siblings. We could not investigate the origin of the deletion in this family because samples from parents of I-1 were not available for testing.

PWS is a classic contiguous gene syndrome that in the majority of affected individuals results from deletion of paternal copies of the imprinted chromosome 15q11–q13 locus.¹⁵ “PW-like syndrome associated with chromosome 6” is another recognized clinical entity (MIM: 176270) that has been described with different genomic deletions of chromosome 6. Of these, some individuals

with interstitial 6q16 deletions most closely resemble the phenotype of PWS. Most such 6q16 deletions encompass *SIM1* (single minded homolog 1 [MIM: 603128]).⁹ *SIM1* is part of the central molecular pathway that regulates body mass. In brief, the adipocyte-derived hormone leptin (*LEP* [MIM: 164160]) and its widely expressed leptin receptor (*LEPR* [MIM: 601007]) stimulate proopiomelanocortin (*POMC* [MIM: 176830]) expression in the hypothalamus.¹⁶ *POMC* is enzymatically cleaved to form α - and β -melanocyte-stimulating hormones, which activate signaling via melanocortin-4 receptor (*MC4R* [MIM: 155541]) to induce expression of *SIM1*. This pathway is dysregulated in multiple monogenic human obesity syndromes (Table S1). Disruptions or heterozygous loss-of-function mutations of *SIM1* cause severe obesity (MIM: 601665).^{10–12} *SIM1* is a master regulator of neurogenesis and its optimum dosage is essential for the formation of supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei that play a central role in body mass regulation.^{17,18} In mice, *Sim1* haploinsufficiency results in neuroanatomical defects but hyperphagic obesity develops even in the absence of structural abnormalities, which is thought to be mediated by deficiency of oxytocin.¹⁹ However, the mechanism of how *SIM1* regulates oxytocin is not known. There is no known conserved binding site for *Sim1* or its heterodimer partner *Amt2* in 5 kb upstream or downstream genomic sequence of *Oxt* (oxytocin).¹⁹ Interestingly, some individuals with PWS-like phenotype have 6q16 deletions that do not encompass *SIM1*.⁹ This suggests that there is at least one other gene located on 6q16, loss of which can cause intellectual disability and obesity and perhaps lies within the leptin > melanocortin > *SIM1* pathway.

The deletion in family 1 encompasses nine known protein-coding genes: *POU3F2* (MIM: 600494), *FBXL4* (MIM: 605654), *FAXC*, *COQ3* (MIM: 605196), *PNISR* (MIM: 616653), *USP45*, *TSTD3*, *CCNC* (MIM: 123838), and *PRDM13* (Figure 1B). Notably, the deletion does not include *SIM1*. Constitutional genomic rearrangements can convey phenotypes through a number of mechanisms including long-range effects.³ For genomic deletions, the most common mechanism is haploinsufficiency of a single dosage-sensitive critical gene or a group of contiguous genes located within the deleted interval. We, therefore, first investigated the likely effect of haploinsufficiency of the genes within the 6q16.1 deletion. Truncating variants have been described in seven of these nine genes in the general population (Table S2). Loss of one copy of any of these single genes, therefore, is less likely to be driving the congenital or childhood-onset phenotypes in family 1. Out of the remaining two genes, population variant frequency data was unavailable for *TSTD3*, and *POU3F2* was the only gene with no known truncating mutations and low haploinsufficiency index score.²⁰ This made *POU3F2* an important candidate for further investigation. Another potential gene of interest was *FAXC* because truncating variants in this gene are extremely rare (Table S2).

We interrogated the local clinical cytogenetic databases of our collaborators for <2 Mb 6q16 deletions that excluded *SIM1* and identified six additional individuals from five families (Figure 1; Table S3). Their clinical features were remarkably similar (Table 1). In four individuals, deletions were proven to be de novo in origin and one individual had inherited the deletion from her similarly affected mother (Figure 1).

Overall, in ten individuals (six males and four females) from six families, we report identification of a disorder of developmental delay and intellectual disability with susceptibility to obesity caused by heterozygous 6q16 deletions that encompass *POU3F2* but do not include *SIM1* (Figure 1; Table 1). Our findings are supported by (1) de novo origin of the deletions in at least one affected member of four families; (2) segregation of the phenotype with the deletion in multiplex families; and (3) phenotypic similarity among affected individuals who were independently ascertained without any bias from clinical features. Birth weights of most individuals were within the normal range. Most individuals presented with neonatal hypotonia, although it was not as severe as what is generally encountered in PWS. Unlike PWS, neonatal feeding difficulty, although encountered, was not a major feature in this group of individuals. Most individuals had mild gross and fine motor delay but one individual had normal motor development and another was severely delayed. Most individuals achieved independent sitting between the ages of 6 and 12 months and independent walking between 14 and 21 months. Most individuals had intellectual disability that ranged from mild to moderate. One individual had severe intellectual disability. The body mass index (BMI) could be calculated for four adults in our cohort and it ranged between 3.62 and 4.59 SDs above the mean, putting them in either severely or very severely obese categories. The BMI of all but one child was on or above the 99th centile (range: +1.51 to +4.27 SDs) in the obese category. The BMI of one 13-year-old girl was on the 91st centile, putting her in the overweight category. The age of onset of obesity ranged from the first year of life to mid-teens. All but one affected individual was reported to have abnormally increased appetite. Interestingly, in all the cases where information was available, the onset of obesity preceded hyperphagia. The excess weight in all individuals was more predominant in the truncal area. Relatives frequently described problems with unpredictable behavior with unprovoked outbursts of aggression, tantrums, impulsivity, mood swings, and emotional lability. Some individuals were described as withdrawn and poor at social interaction. Interestingly, a recent genome-wide association study revealed 6q16.1 as a risk locus for bipolar disorder.²¹

A combined analysis of all the deletions revealed that the maximum critical region for the phenotype included only two genes, *POU3F2* and *FBXL4* (Figure 1B). Loss-of-function and truncating *FBXL4* mutations cause autosomal-recessive encephalomyopathic type mitochondrial DNA

Table 1. Clinical Features of Individuals in This Study

Individual#	Pedigree Identifier ^a	Inheritance	Sex	Age	Birth weight in kg (centile)	Neonatal Hypotonia	Feeding Difficulties in Infancy	Motor Delay	Speech Delay	Learning Disability
1	1-I-1	NA	male	61	NA	yes	NA	yes	yes	moderate
2	1-II-3	paternal	male	21	4.8 (98 th)	yes	no	mild	yes	mild
3	1-II-4	paternal	male	19	4.3 (91 st)	yes	no	mild	yes	moderate
4	1-II-6	paternal	male	10	3.5 (50 th)	no	no	mild	yes	moderate
5	2-II-1	DN	female	13	3.4 (50 th)	no	no	no	yes	moderate
6	3-II-2	DN	female	52	3.25 (50 th)	yes	no	mild	moderate	mild
7	3-III-1	maternal	female	26	3.1 (25 th)	yes	no	mild	mild	mild
8	4-II-1	DN	male	9	3.69 (83 rd)	yes	no	mild	mild	mild
9	5-II-1	DN	female	4	3.3 (75 th)	yes	yes	severe	severe	severe
10	6-II-1	NA	male	16	3.5 (50 th)	yes	yes	moderate	severe	moderate

Abbreviations are as follows: DN, de novo; NA, not available; IQ, intelligence quotient; WISC, Wechsler Intelligence Scale for Children.

^aPedigree identifier: Family#-Generation#-individual# (compare with Figure 1).

^bBMI categories for adults are 25 to 30, overweight; 30 to 35, moderately obese; 35 to 40, severely obese; over 40, very severely obese.

^cBMI categories for children are 85th to 95th centile, overweight; above 95th centile, obese.

depletion syndrome (MIM: 615471) in which heterozygous carriers are phenotypically unaffected.²² Thus *POU3F2* remained as the most likely critical gene for the phenotype of 6q16.1 deletions.

Class III POU genes, *POU3F1* (MIM: 602479), *POU3F2*, *POU3F3* (MIM: 602480), and *POU3F4* (MIM: 300039), belong to a family of transcription factors that bind to the octameric DNA sequence 5'-ATGCAAAT-3'. These genes share a highly homologous POU domain and are predominantly expressed in the central nervous system. *Pou3f2* and *Pou3f3* upregulate proneuronal genes^{23,24} and are required for production, migration, and positioning of neocortical neurons.^{25,26} In vivo, *Pou3f2* shares functional redundancy with *Pou3f3* and simultaneous disruption

of both genes is required to disturb normal formation of the neocortex and migration of neurons.²⁶ However, *Pou3f3* single mutants exhibit an abnormal hippocampus²⁶ and *Pou3f2* single mutants display abnormal neurosecretory neurons of the hippocampal PVN and SON^{27,28} thus demonstrating their essential roles in the development of specific areas of the brain.

We examined data from the Human Brain Transcriptome Project²⁹ that confirmed that *POU3F2* is expressed throughout fetal and adult life in the human brain (Figure S1). We then examined data in the Allen Human Brain Atlas³⁰ via the Brain Explorer tool for expression patterning of *POU3F2* and related genes in the adult hypothalamus and hippocampus (Figure S2). This confirmed

Behavioral Issues	Latest Weight in kg (centile)	Latest Height in cm (centile)	Latest BMI (SD)	BMI Category	Obesity Onset (years)	Hyperphagia	Hyperphagia Onset (years)	Other Comments
mood swings, occasional aggressiveness	NA	172 (25 th)	NA	NA	NA	yes	NA	diabetes mellitus at 55 years, angioplasty at 60 years
mood swings, acute depression, occasional aggressiveness	117.8 (>99 th)	177.3 (50 th)	37.5 (3.77)	severely obese ^b	15	yes	19	right hydrocele, undescended testis, and inguinal hernia; left convergent squint
withdrawn, occasional aggressiveness	140.0 (>99 th)	172 (25 th)	47.3 (4.59)	very severely obese ^b	10	yes	12	left convergent squint
tantrums, emotional lability	59.5 (> 99 th)	139 (50 th – 75 th)	30.8 (4.27)	obese ^c	1	yes	8	left undescended testis, right hydrocele; right talipes; convergent squint
poor social interaction, occasional aggressiveness	57 (75 –90 th)	160 (75 th)	22.3 (1.51)	overweight ^c	NA	no	NA	partial regression of speech after 2 years; at 13 years: WISC-III total IQ = 48, verbal IQ = 45; performance IQ = 62
tantrums, rigid behavior	101 (95 th)	161 (25 th)	38.9 (3.87)	severely obese ^b	19	yes	21	clumsiness and poor articulation; lordosis; hyperhidrosis.
rigid behavior, occasional aggressiveness	95 (NA)	160 (NA)	37.1 (3.62)	severely obese ^b	11	yes	14	hypothyroidism; systemic lupus erythematosus-like illness
impulsiveness, occasional aggressiveness	66 (99 th)	152.5 (99 th)	28.38 (3.57)	obese ^c	4	yes	8	IQ = 72 (7.5 years)
sleep problems	24.6 (>97 th)	109.5 (98 th)	20.7 (2.99)	obese ^c	2	yes	NA	gastro-esophageal reflux; very poor expressive speech; thin corpus callosum; kyphosis
occasional aggressiveness poor social interaction	88.9 (99 th)	184 (91 st)	26.3 (2.29)	obese ^c	10	yes	NA	auditory memory problems and dyspraxia; left cerebellar arachnoid cyst

that *POU3F2* and *POU3F3* are highly expressed in the human hypothalamus and hippocampus, respectively (Figure S2), thus mirroring the known expression pattern in mice.

We also explored the expression of *POU3F2* through quantitative RT-PCR (qRT-PCR) in transformed lymphoblastoid cell lines (LCLs) from affected individual II-6 and his unaffected sibling II-1 in family 1. Amplification of the *POU3F2* transcript occurred at very late cycle numbers, suggesting that this gene is expressed at very low levels in peripheral lymphocytes. We observed an approximate 50% reduction in *POU3F2* expression in LCLs from the affected individual in comparison to cells derived from the unaffected sibling (Figure S3), although the results

were not statistically significant (most likely due to the low expression levels resulting in high variability) even after nine technical repeats and by using a high concentration of cDNA template.

We explored the role of *POU3F2* in hypothalamic development using zebrafish models. We determined that zebrafish have two orthologs of human *POU3F2*, *pou3f2a* and *pou3f2b* (Figures S4 and S5 and Table S4). All procedures were in accordance with NIH guidelines on the care and use of animals and were approved by the Georgetown University Institutional Animal Care and Use Committee, Protocol 11-008. Zebrafish (*Danio rerio*) were raised, maintained, and crossed as described previously.³¹ Embryos were raised at 28°C and staging was determined by

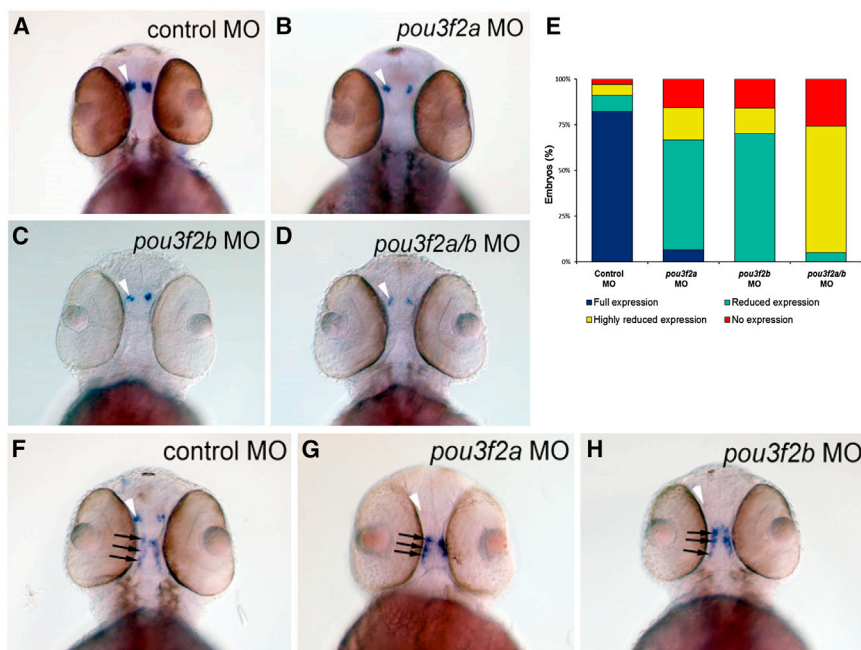


Figure 2. Effect of *pou3f2a* and *pou3f2b* Morpholino Oligonucleotides Knockdown on *oxt*- and *avp*-Expressing Cells

Representative ventral views of 48 hpf embryos stained for *oxt* (A–D) and *avp* (F–H) expression by whole mount in situ hybridization (WISH). The white arrowheads indicate the location of neuroendocrine preoptic area (NPO) and black arrows indicate *avp* expression in the ventral hypothalamus.

(A) Control MO showing full *oxt* expression (n = 67).

(B) *pou3f2a* MO showing reduced *oxt* expression (n = 45).

(C) *pou3f2b* MO showing reduced *oxt* expression (n = 69).

(D) *pou3f2a/pou3f2b* MO-injected embryos showing highly reduced *oxt* expression (n = 96).

(E) Quantification of *oxt* expression. 82% of control MO-injected embryos had full *oxt* expression (blue). Injection of either *pou3f2a* or *pou3f2b* MO resulted in majority of the embryos with reduced *oxt* expression (green). Simultaneous injection of *pou3f2a* and *pou3f2b* MOs resulted

in highly reduced *oxt* expression (yellow) majority of the embryos with 26% showing no expression (red). (F) Control MO showing full *avp* expression within the NPO and ventral hypothalamus.

(G and H) *pou3f2a* (G) and *pou3f2b* (H) MO showing no *avp* expression within the NPO without any reductions in its expression in the ventral hypothalamus.

both hours post fertilization (hpf) and morphological characteristics.³²

Plasmids for *pou3f2a* and *pou3f2b* were obtained from RZPD (German Science Centre for Genome Research). The *pou3f2a* DIG-labeled antisense riboprobe was generated using T7 polymerase from RsrII linearized plasmid (DIG-labeling Kit, Roche). For *pou3f2b*, the cDNA was subcloned into pBluescript II (Stratagene) and then linearized with KpnI for riboprobe synthesis using T7. Single- and double-labeled whole mount in situ hybridization (WISH) was performed according to previously published protocol.³³ We determined that, in zebrafish, by 48 hpf, *pou3f2a* and *pou3f2b* are normally expressed in the diencephalon, the midbrain tegmentum, and throughout the hindbrain (Figure S6). However, within the diencephalon, strong expression of *pou3f2a* and *pou3f2b* remains restricted to a small area of the neuroendocrine preoptic area (NPO). Double-labeled WISH demonstrated that in the NPO, *pou3f2a* and *pou3f2b* mRNAs normally co-localize in subsets of *oxt*-expressing cells (Figure S7).

Antisense morpholino oligonucleotides (MOs) targeting *pou3f2a* or *pou3f2b* (Table S5) were injected independently or simultaneously into zebrafish embryos at the 1- to 2-cell stage in 1× Danieau's solution at 1.0 ng/embryo. The total amount of injected MO in each group was kept constant for each embryo. *Oxt* and *avp* probes were generated as previously described.^{33–35} *Oxt* expression in approximately 30 cells was quantified as full expression, in 5–15 cells as reduced expression, in 1–4 cells as highly reduced expression, and in 0 cells as no expression. Antisense

MO-mediated knockdown of *pou3f2a* or *pou3f2b* individually resulted in significantly decreased *oxt* expression (Figure 2). Simultaneous knockdown of *pou3f2a* and *pou3f2b* decreased *oxt* expression further (Figure 2), demonstrating the role of *POU3F2* in regulating *OXT* expression. Likewise, *pou3f2a* or *pou3f2b* MOs individually eliminated *avp* expression within the NPO. However, neither *pou3f2a* nor *pou3f2b* MOs reduced *avp* expression in the ventral hypothalamus, demonstrating the specificity of the MOs.

We have previously shown that MO knockdown of *sim1a* eliminates *oxt* and *avp* expression in zebrafish NPO.^{33,34} Additionally, oxytocin expression is decreased in *Sim1* haploinsufficient mice¹⁹ and there is evidence that *Sim1* might regulate *Pou3f2* expression in mice.¹⁷ MO injections for *sim1* were performed as previously published^{33,36} and WISH staining for *pou3f2a/b* expression in the NPO was quantified using an ordinal scale from 0 to 2 as follows: 0, no staining; 1, uncertain or dramatically reduced staining; 2, obvious (normal) staining. This showed significant reduction of *pou3f2a* and *pou3f2b* expression levels in *sim1* morphants (Figures 3A–3D and 3I). *ARNT2* encodes a dimerization partner of *SIM1* for the development of the hypothalamus.³⁷ In the NPO of previously described³⁸ homozygous *arnt2*^{hi2639Tg} null zebrafish mutant embryos (Table S6), the expression of *pou3f2a* and *pou3f2b* was undetectable (Figures 3E–3H and 3J), demonstrating the role of *SIM1*-*ARNT2* dimers in regulating *POU3F2* expression in the hypothalamus. We also examined the expression of *sim1a* in NPO of *pou3f2a* and *pou3f2b* morphants and found no obvious differences from controls (Figure S7).

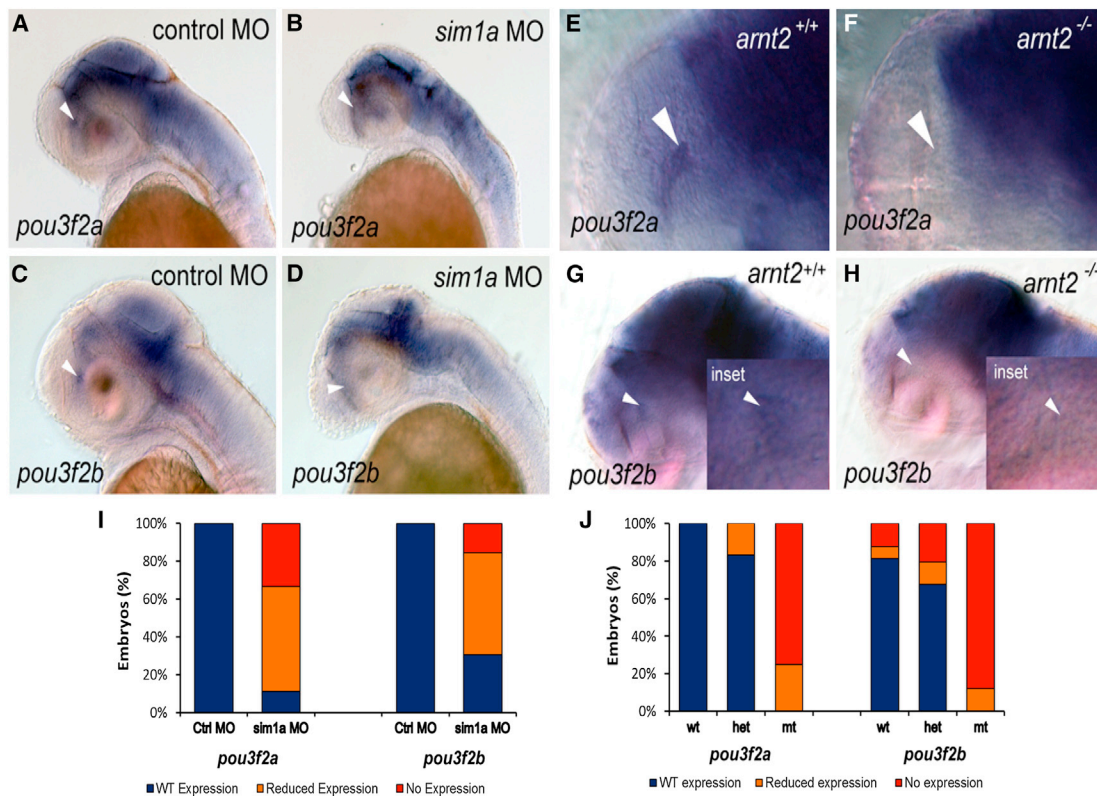


Figure 3. *pou3f2a* and *pou3f2b* Expression Is Reduced in *sim1a* Morphants and Is Eliminated in *arnt2*-Null Mutant Embryos

Representative lateral views of embryos stained for *pou3f2a* or *pou3f2b* expression by WISH at 48 hpf. Eyes have been removed to better visualize the staining in the NPO (indicated by white arrowheads and magnified views are shown in the insets).

(A and C) Control morpholino oligonucleotide (MO)-injected embryos showing normal expression of *pou3f2a* ($n = 11$) (A) and *pou3f2b* ($n = 10$) (C).

(B and D) *sim1a* MO knockdown reduces the level of *pou3f2a* ($n = 9$) (B) and *pou3f2b* ($n = 12$) (D) expression.

(E and G) Wild-type embryos showing strong *pou3f2a* ($n = 7$) (E) and *pou3f2b* ($n = 13$) (G) staining in the NPO.

(F and H) *arnt2*-null mutant embryos showing an absence of *pou3f2a* ($n_{wt} = 7$; $n_{het} = 12$; $n_{hom} = 8$) (F) and *pou3f2b* ($n_{wt} = 13$; $n_{het} = 28$; $n_{hom} = 18$) (H) in the NPO.

(I and J) Both *sim1* MO-injected embryos (I) and *arnt2*-null mutants (J) resulted in a significant number of embryos showing reduced (orange) or no (red) expression of *pou3f2a* and *pou3f2b* indicating that their expression in the NPO is dependent on functional *sim1a*-*arnt2* heterodimers.

Overall, the zebrafish experiments showed that *POU3F2* is a downstream target for the *SIM1*-*ARNT2* dimer in the leptin > melanocortin > *SIM1* pathway and that *POU3F2* plays an important role in regulating expression of *OXT* in the hypothalamus.

The maximum critical region for the 6q16.1 deletions and our zebrafish work suggests that the phenotype of the individuals described here is due to haploinsufficiency of *POU3F2*. Mice that are homozygous for loss-of-function *Pou3f2* mutations die within 10 days of birth, whereas heterozygous mice have half-the-normal levels of vasopressin and oxytocin in the hypothalamus in comparison with the wild-type mice.²⁷ This suggests that haploinsufficiency of *POU3F2* might affect hypothalamic development or functions more specifically than other brain regions. The hypothalamus is a critical integrator of neural and humoral signals that has, among its numerous functions, a fundamental role in controlling the body's energy expenditure, food intake, social behavior, learning, and memory.

Abnormal development, survival, or function of hypothalamic neurons is known to underpin a number of disorders associated with obesity, hyperphagia, and abnormal neurodevelopment.¹⁶ This further supports the putative role of *POU3F2* deletion in the phenotype seen in the individuals described here. The similarity of the clinical features described here with those caused by loss-of-function *SIM1* mutations^{11,12} is in keeping with our conclusions. However, an interesting difference in individuals presented here is that hyperphagia was reported to develop after the onset of obesity. The underlying reason or mechanism for this is not clear and will need identification of younger pre-symptomatic individuals in the future to confirm this observation.

The intellectual disability and neuropsychological problems associated with haploinsufficiency of *POU3F2* and *SIM1* might result from decreased oxytocin levels. Oxytocin is required for activity-dependent cortical development and cortical plasticity³⁹ and is known to shape social learning⁴⁰

and emotional responses. Future studies of disorders of this pathway could help delineate the precise function of oxytocin in human learning and behavior. Alternatively, the phenotype might also be due to effects that are independent from the role of oxytocin. For example, *POU3F2* is known to regulate *FOXP2*, mutations in which cause speech-language disorder-1 (MIM: 602081).^{41,42}

The phenotypic variability among individuals presented here is notable. For example, the phenotype observed in individual 5 (family 2, individual II-1 in Figure 1) is significantly milder than the clinical features of individual 9 (family 5, individual II-1 in Figure 1). The reason behind this is not clear. It could be due to the differences in the size of their deletions—individual 5 has the smallest deletion that includes only two genes (*POU3F2* and *FBXL4*) and individual 9 has the largest deletion that includes *MCHR2* in addition to all the nine genes deleted in family 1. Alternatively, genetic background or environmental factors might influence the phenotype.

It is remarkable that *SIM1* regulates *POU3F2* and, in humans, both genes are located on 6q16.1, less than 1.6 Mb apart. This raised the possibility that *SIM1* and *POU3F2* might share common regulatory regions, deletion of which might have an effect on the 6q16 deletion phenotype. To test this hypothesis, we examined the evolutionary syntenic architecture of the region using Genomicus browser,⁴³ which showed that *SIM1* and *POU3F2* are located on different chromosomes in a number of species, including mice (Figure S9). This reduces the probability of shared regulatory regions between *SIM1* and *POU3F2* on 6q16.1.

Notably, we have not described any individuals with *POU3F2* point mutations. Hence, a more complex mechanism, such as one involving long-range gene dysregulation, cannot be completely ruled out to underlie the phenotype seen with 6q16.1 deletions. It will be interesting to see whether nonsense or loss-of-function *POU3F2* mutations can result in the same phenotype. This question might be best answered by targeted sequencing or interrogating exome sequencing data from large cohorts of individuals with ID or obesity. We interrogated data from more than 4,000 trios in the DDD study⁴⁴ and did not find any truncating *POU3F2* mutation in this cohort. Intellectual disability and obesity both are listed phenotypes in approximately 3% of probands in the DDD study (C. Wright, personal communication). Of note, *POU3F2* is composed of a single exon (Figure S10). Thus, truncating mutations in this gene might not lead to nonsense-mediated decay and, therefore, might not necessarily result in haploinsufficiency. Second, the *POU3F2* sequence is extremely GC rich (Figure S10) and, therefore, accurate sequencing might be challenging. Examination of coverage metrics for *POU3F2* in the ExAC database revealed extremely poor coverage for almost 50% of the gene (Figure S10).

In summary, we have described overlapping 6q16.1 deletions in ten individuals, from six families, with variable developmental delay, intellectual disability, and susceptibility to obesity and hyperphagia. The likely mechanism is haploinsufficiency of *POU3F2*. Our work helps to further define the neuro-endocrine control of energy balance/food intake and its role in human monogenic obesity by demonstrating that *POU3F2* functions downstream to *SIM1* in the leptin > melanocortin > *SIM1* > oxytocin pathway and is an important mediator of the clinical and biochemical effects (decreased oxytocin levels) of loss of *SIM1* activity. Our zebrafish work, previous work on mouse models,^{17–19,27,28} the human phenotypes, and our analysis of the expression patterning of these genes demonstrate that the molecular pathway linking genes related to hypothalamic function is conserved across species, emphasizing its biological importance.

Supplemental Data

Supplemental Data include ten figures and six tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2015.12.014>.

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Web Resources

The URLs for data presented herein are as follows:

Allen Human Brain Atlas, <http://human.brain-map.org/>

COBALT: Multiple Alignment Tool, http://www.st.va.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi

DECIPHER, <http://decipher.sanger.ac.uk/>

ExAC Browser, <http://exac.broadinstitute.org/>

Genomicus v80.01, <http://www.genomicus.biologie.ens.fr/genomicus-80.01/cgi-bin/search.pl>

HBT – Human Brain Transcriptome, <http://hbatlas.org/>

MUSCLE, <http://www.ebi.ac.uk/Tools/msa/muscle/>

OMIM, <http://www.omim.org/>

Syntenic Database, http://syntenydb.uoregon.edu/syntenic_db/

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